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Original Research Article

Does Surgical Treatment Affect Serum Levels of Vascular Endothelial Growth Factors (VEGF) in Orofacial Tumours?

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Abstract

Vascular Endothelial Growth Factor (VEGF) is closely related to tumour progression and prognosis. This study aims to evaluate the pattern and clinical implication of VEGF expression in benign and malignant neoplasms and to determine the effect of surgery on serum level of VEGF. Cases were drawn from among histologically confirmed orofacial tumours, while controls were from consecutive, ambulatory dental patients at the University of Benin Teaching Hospital. Blood samples taken from cases and controls, preoperatively and at one -month post-surgery, were allowed to clot and the serum aliquots stored at -60^{0}C till analyses. Serum VEGF was analysed using Sandwich ELISA and mean levels as well as median were measured. Spearman correlation was estimated, t-test measured significance (at $p \leq 0.05$). Eighty-one subjects were studied (55 cases and 26 controls). Preoperatively, there were significantly different serum levels of VEGF in benign (48.11 ± 25.19 pg/ml) vs. malignant cases (1065.00 ± 412.14 pg/ml), and when compared with controls (45.42 ± 29.83 pg/ml), p <0.001. After definitive surgical intervention, there was a significant reduction in the level of serum VEGF in both benign (45.50 ± 24.71 pg/ml) and malignant cases (51.22 ± 16.84 pg/ml), p <0.001. One-way ANOVA revealed no significant differences between cases and controls post-surgery. There was elevated levels of serum VEGF in benign and malignant orofacial neoplasia. Surgery caused a significant reduction of circulating VEGF to normal levels irrespective of age, gender, previous medical history, perceived duration of the lesion and lymph node involvement.
Keywords: VEGF, orofacial tumour, surgery.

INTRODUCTION

Angiogenesis occurs in utero, in physiological situations such as pregnancy and exercise, and in pathological conditions. However, the angiogenesis induced by solid tumours differs from that provoked by conditions such as trauma and inflammation. This is because tumour induced angiogenesis is not self-limiting and continues indefinitely until eradication of the tumour or death of the host [1].

Angiogenesis is regulated by several factors such as fibroblast growth factors (FGFs), transforming growth factors (TGFs), insulin-like growth factor 1(IGF-1) [2], vascular endothelial growth factor (VEGF), although VEGF has generated the most interest as its angiogenic role is unique and pivotal [3].

VEGF is a highly specific mitogen for vascular endothelial cells [4] and plays a role in transcapillary permeability, stimulates cell differentiation, proliferation, migration, and survival [5]. VEGF levels are elevated in physiologic conditions such as exercise, pregnancy, wound healing, and in disease conditions such as solid tumours and haematolymphoid malignancies [6-9].

The stimulant for VEGF production in tumours appears to be hypoxia as in normal cells [10]. As the tumour size increases, there is induction of vascular development for adequate oxygen and nutrient supply. If this supply is insufficient, hypoxia results and stimulates angiogenesis through the up-regulation of Hypoxia-Inducible Factor-1 alpha (HIF-1 alpha) and VEGF [11, 12]. The VEGF thus produced leaks into the bloodstream because of a concomitant increased permeability of the blood vessels. Hypoxia up-regulates VEGF production by increasing mRNA transcription and increasing its stability [13] via Hypoxia Inducible Factor-1 (HIF-1) [14]. The importance of HIF-1a in tumour angiogenesis is further accentuated by the demonstration of in vivo reduction of tumour growth and neovascularization where there is functional impairment of HIF-1a [15].

The lungs, kidneys, heart, and adrenal glands are the dominant sites of expression of the VEGF gene in healthy adult animals [16], though other tissues in the body have the potential to produce VEGF when stimulated [17]. VEGF produced is secreted into the circulation by paracrine means. The serum VEGF level has been found to be higher because of storage of VEGF in the alpha granules of platelets [18]. During clotting, there is release of these granules leading to an increase of VEGF in the sample. In the normal healthy population, the plasma VEGF levels have been found to range between 0–8 pg/ml [19]. Serum levels on the other hand are usually higher, ranging from 17–287 pg/ml as reported in a meta-analysis of VEGF distribution in cancer [20].

In tumours of the aero digestive system, salivary VEGF may be a source of as much prognostic information as serum VEGF. However, the values may be affected by concurrent inflammatory processes, and ulceration in the mouth [21]. Salivary VEGF has not been widely studied as serum and plasma VEGF and may not be a very reliable tool currently for measuring neovascularization in head and neck tumours.

Treatment of tumours of the orofacial region is a challenge mainly because of the daunting task of reconstruction as well as the possibility of recurrence. Currently, loco-regional treatment (surgery alone or surgery and radiotherapy) is still the mainstay of treatment of malignant tumours of the head and neck [22, 23]. Nevertheless, there is need for systemic adjuvant where there is disseminated disease [24]. Benign tumours on the other hand are amenable to surgery alone. Despite the successes achieved with the conventional therapies, early detection of tumours [25], adequate monitoring and follow-up after treatment will help improve the treatment outcome and reduce the morbidity associated with management of orofacial tumours.

Consequently, the use of a sensitive surveillance tool such as VEGF will help in the early detection of orofacial tumours. Furthermore, an objective follow-up can be instituted by observing for the decay of previously elevated markers in the internal environment. This will aid in patient's reassurance, qualifying outcome of surgery and timing of reconstructive surgeries.

It is hoped that the outcome of this study will increase the available body of knowledge on VEGF as it seeks to improve the decision-making process in caring for cancer patients.

MATERIALS AND METHODS

This study was carried out at the Department of Oral and Maxillofacial Surgery, University of Benin Teaching Hospital (UBTH), between October 2015 and October 2017. Written informed consent was obtained from all participants and the institution's ethics and research board approved the study (CMS/RES/O1/VOL 004).

Inclusion criteria for cases were consenting adults above 18 years who had histologic diagnosis orofacial tumour according to the WHO 2005 classification [26]. Exclusion criteria included patients with secondaries from a distant primary site that was not orofacial and those who had a previous history of surgical treatment of a tumour of other parts of the body of less than 5 years duration.

Fifty-five cases histologically diagnosed of any orofacial neoplasm and 26 controls drawn from consecutive healthy patients attending the maxillofacial clinic were recruited for the study.

The cases comprised two groups (malignant and benign lesions) based on preoperative histologic diagnosis. The controls were matched for age and sex and were used to compare findings with both the benign and malignant group of cases.

The biodata, history of presenting complaint and the medical history of each patient were recorded. Malignant lesions were staged clinically according to the TNM system. Lymph nodes were assessed clinically to determine involvement.

Sample collection and storage (Serum VEGF)

Intravenous blood (5mls) was obtained from all subjects using plain bottles and allowed to clot for 1 hour before centrifuging at 1200 x g. Serum was aliquoted and stored at -60^{0}C. Samples were collected from the subjects on the operating table before the first incision and at one month after definitive surgery had been done to ascertain the pre-operative and post-operative VEGF levels. Samples were collected only once from the control cohort.

Serum samples were analysed for VEGF concentration (pg/ml) using sandwich Enzyme Linked Immunosorbent Assay (ELISA) [Eagle Biosciences Inc. (Nashua, NH, USA)]. Absorbance was read at 450nm by an automated ELISA reader [SPECTRAmax^{TM} 340 Microplate Reader (Sunnyvale, CA, USA)] with a reference wavelength of 690nm. A standard curve for VEGF levels was established by taking the logarithmic values of the standard VEGF specimens as axis Y and the relative absorbance values as axis X ($r=0.951$, $p=0.004$). Each specimen from the patients and healthy blood donors was measured twice for VEGF concentrations and the levels of VEGF in the specimens were determined from the plot of the standard curve.

Data Management

Spearman correlation showing the relationship between VEGF in serum in benign and malignant cases, and Kruskal-Wallis one-way ANOVA measuring the

difference in serum VEGF before and after surgery was done.

RESULTS

There were 35 (43.2%) males and 46 (56.8%) females with a male to female ratio of 1:1.3. The control group and test group had similar gender characteristics (p = 0.713). The age range of the study participants ranged was 21-73 years with a mean age of 40.35 ± 14.91 years (Table-1).

Table-1: Socio-demographic characteristics of the participants

Variables	Study group			
	Cases (n = 55) n (%)	Controls (n = 26) n (%)	Total (n = 81) n (%)	p-value
Sex				
Male	23 (41.8)	12 (46.2)	35 (43.2)	0.713
Female	32 (58.2)	14 (53.8)	46 (56.8)	
Total	55 (67.9)	26 (32.1)	81 (100.0)	
Age group (years)*				
20 – 29	19 (34.5)	9 (34.6)	28 (34.6)	0.961
30 – 49	22 (40.0)	11 (42.3)	33 (40.7)	
50 – 69	11 (20.0)	5 (19.2)	16 (19.8)	
70+	3 (5.5)	1 (3.8)	4 (4.9)	
Total	55 (67.9)	26 (32.1)	81 (100.0)	

**Mean (sd): 40.35 (14.91) years*

Table-2 shows the age and sex characteristics of the cases. The test group had 35 (63.6%) benign and 20 (36.4%) malignant neoplasia. The gender distribution between the subjects with benign and malignant tumours was similar (p = 0.836) with 23 (41.8%) males and 32 (58.2%) females in a ratio of 1:1.4. Both groups had more females than males. The age ranged from 21 years to 73 years with a mean age of approximately 40.40 ± 15.59 years. Subjects with benign tumour were mainly between the 3rd and 5th decade (88.6%) while those with malignancy were between 4th and 7th decade (75%). The most prevalent malignant neoplasms were squamous cell carcinoma (60%) while ameloblastoma (82.9%) accounted for most of the malignant cases that presented (Table-3).

Table-2: Socio-demographic characteristics of the participants with tumour

Variables	Types of tumour cases			p-value
	Benign (n = 35) n (%)	Malignant (n = 20) n (%)	Total (n = 55) n (%)	
Gender				
Male	15 (42.9)	8 (40.0)	23 (41.8)	0.836
Female	20 (57.1)	12 (60.0)	32 (58.2)	
Total	35 (63.6)	20 (36.4)	55 (100.0)	
Age group (years)*				
20 – 39	22 (62.9)	5 (25.0)	27 (49.1)	0.002
40 – 59	11 (31.4)	7 (35.0)	18 (32.7)	
60 – 79	2 (5.7)	8 (40.0)	10 (18.2)	
Total	35 (63.6)	20 (36.4)	55 (100.0)	

**Mean (sd): 40.40 (15.59) years.*

Table-3: Frequency distribution of types of tumour

Type of tumour	n	%
Malignant tumour		
Squamous cell carcinoma	12	60
Adenoid cystic carcinoma	4	20
Adenocarcinoma	3	15
Mucoepidermoid carcinoma	1	5
Benign tumour		
Ameloblastoma	29	82.9
Pleomorphic adenoma	3	8.6
Odontogenic myxoma	2	5.7
Adenomatoid odontogenic tumour	1	2.8

There was an overall elevation of serum VEGF in the case group with a mean serum level of 418.2 ± 551.6pg/ml compared to the control group with a mean level of 45.4 ± 29.8pg/ml. (Figure-1). The difference in the level of expression was statistically significant (p <0.001).

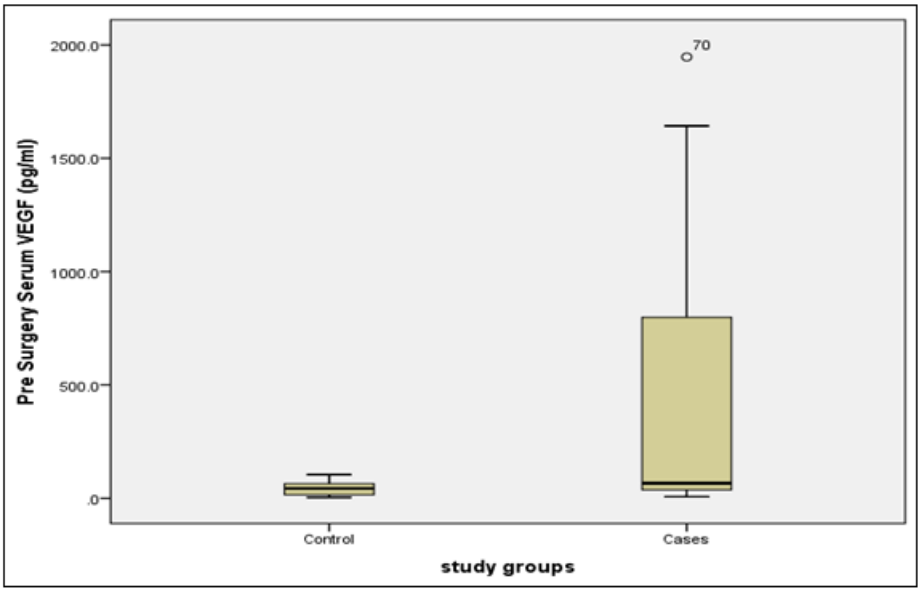

Fig-1: Comparison of serum VEGF between cases and controls

A comparison of the serum levels of VEGF between the malignant and benign tumours is shown in Figure-2. The serum VEGF level $_{ranged}$ from 7.2 to 102.7pg/ml with a mean of 48.1 ± 25.2pg/ml in subjects with benign tumour while the serum VEGF level in subjects with malignant neoplasms ranged from 436.8 to 1946.9pg/ml with a mean of 1065.7 ± 412.1pg/ml. This difference was a statistically significant (P < 0.0001).

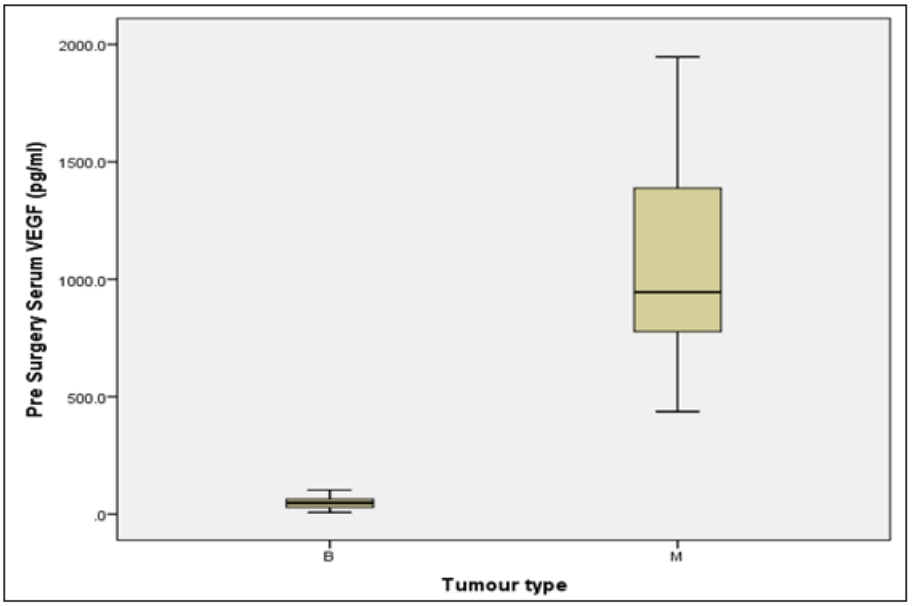

Fig-2: Comparison of serum VEGF among the participants with benign and malignant tumour (B= benign; M= malignant)

Table-4 showed that the serum VEGF was higher pre-surgery than post-surgery. There was a statistically significant drop in the level of circulating VEGF following surgery (P <0.0001). Post-surgery VEGF ranged from 6.10 to 96.70 pg/ml for the benign cases while the post-surgery VEGF for malignant cases ranged from 11.80 to 77.60 pg/ml.

Table-4: Mean serum VEGF level for pre and post-surgery by tumour type

Tumour type	Serum VEGF Mean (SD) pg / ml	p-value
Benign		
Pre surgery	48.11 (25.19)	<0.001
Post-surgery	45.50 (24.71)	
Malignant		
Pre surgery	1065.74 (412.14)	<0.001
Post-surgery	51.22 (16.84)	

The VEGF levels of post-surgical tumour cases and those of the control cohort, were found to be comparable. There was no significant difference between controls, benign and malignant groups (Table-5).

Table-5: Effect of surgery on serum VEGF of the cases compared to normal healthy controls

	Post-surgery VEGF Mean (SD) pg /ml	Post-surgery VEGF Median (IQR)
Control	45.42 (29.83)	43.45 (15.38 – 64.55)
Benign	45.50 (24.71)	46.00 (22.10 – 61.10)
Malignant	51.22 (16.84)	50.95 (40.28 - -67.35)

Kruskall-Wallis test; p= 0.631

There was no statistically significant impact of age, gender, previous medical history, presence of lymph node involvement or the duration of the lesion on the difference in pre- surgical and post-surgical serum VEGF using multiple linear regression as shown in Table 6. Overall significance value was 0.676.

Table-6: Multilinear regression for predictors of VEGF changes following surgery

Variables	B		Sig.
Constant	926.95		0.348
Age	6.04		0.535
Gender	148.87		0.621
Medical History	-0.26		0.996
Presence of Lymph Node	-279.33		0.273
Duration of Lesion	-42.72		0.286

P=0.676

DISCUSSION

Previous studies have reported a higher male preponderance for head and neck tumours [27, 28]. The finding of this study is however contrary with a male to female ratio of 1:1.3 but with no statistically significant difference. There is a growing trend in the incidence of head and neck cancer in females owing to changes in the social norms among females possibly explaining the preponderance of females in this study. Furthermore, females have been reported to have better health seeking behaviour [29]. Majority of the participants in this study were within the 2nd and 4th decade of life. This finding was in agreement with most literature on the preponderance of tumours occurring between the 2nd to 4th decade of life [27, 28].

In this study, there was an overall elevation of serum VEGF in orofacial neoplasia compared to the control group. The difference in the level of expression between the control and cases was statistically significant (p <0.001). This is consistent with the literature that report increased expression of VEGF in patients with tumours [31, 32] irrespective of tumour type compared to the normal population [31-33].

Although serum VEGF expression was higher in participants with benign tumours than in the controls the difference was not statistically significant. The serum VEGF level in subjects with malignancy accounted for the observed statistical significance when the overall serum VEGF levels of participants with orofacial tumour was compared with that of controls. These findings are in consonance with previous studies [34, 35] that showed multiple fold increase of serum VEGF in patients with tumour compared to between normal patients. This finding is probably due to the very high rate of cell division and proliferation seen in malignancy compared to benign and normal tissues resulting in greater demand for nutrition as well as reducing the ambient oxygen saturation leading to hypoxia. The combination of these factors provokes an increased angiogenesis [2, 4], and consequent up-regulation of VEGF which is secreted into the circulation and accounts for the tremendous increase in circulating levels especially in malignancies [36, 37].

This study showed that the serum VEGF was higher pre-surgery than post-surgery. The mean serum VEGF pre surgery was 418.16 ± 551.58 but it dropped to 47.58 ± 22.18 post-surgery. Surgery impacted a statistically significant change in the level of circulating

VEGF among all subjects with orofacial tumour ($p<0.001$). Post hoc analysis showed that the post-surgical serum VEGF for benign and malignant tumours was similar to that of the normal healthy controls.

The drop in circulating VEGF following surgery could be as a result of the ablation of the tumour which served to stimulate angiogenesis and increased VEGF secretion. With complete removal of the source of increased circulating VEGF the serum level was expected to drop close to, or within the range of normality as in otherwise healthy control subjects.

Although multiple linear regression showed that age, gender, perceived duration of the lesion, previous medical history and presence of lymph nodes were insignificant ($p=0.676$) as predictors of the change in serum VEGF levels following definitive surgery, gender and presence of lymph nodes was found to have a seemingly higher predictive value (B= 148.87, 279.33 respectively).

The correlation between pre and post-surgery serum VEGF among patients with benign and malignant tumour was strongly positive and statistically significant. This meant that the subjects with the highest VEGF levels experienced the most reduction in VEGF following surgery. This will suggest that it might be possible to use post-surgery VEGF to monitor patients with orofacial tumours for possible recurrence or incomplete tumour excision.

Research on the clinical relevance of VEGF is on the rise and it is hoped that there will be more positive outcomes geared towards the improvement of surveillance, reduction of current ablative treatment measures, and control of the progression of tumours in the head and neck region.

In conclusion, there was a marked impact of surgery on the level of circulating VEGF thereby rendering it as a possible tool for monitoring patients who are being treated for both benign and malignant neoplasms. Based on the significant reduction of the circulating VEGF to similar levels with the normal healthy controls, the null hypothesis is rejected in this study. The determined cut-off for normal healthy controls this environment is 105pg/ml (Mean ± 2SD). Serum VEGF levels greater than this cut-off should arouse the suspicion of a clinician to investigate further.

Conflict of Interest

No conflict of interest.

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